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Assessment of Experimental and Natural Viral Aerosols

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INTRODUCTION

This report describes a number of characteristics of artificially prepared aerosols containing coxsackievirus A, type 21, a virus that causes respiratory illness in man. Studies on natural aerosols produced by subjects who have been infected with this virus are also described. The findings are part of a continuing program of investigation of the role of aerosols in human viral respiratory disease conducted as a joint undertaking by the U.S. Army Biological Center, Fort Detrick, Frederick, Md., and the Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

The report is divided into two sections. The first deals with observations on the properties of laboratory-generated viral aerosols used for inoculation purposes, and the second covers the production of viral aerosols by experimentally infected subjects and the contamination of air in rooms occupied by them.

The program has availed itself of a large body of information concerning bacterial aerosols and was aided by some new techniques pertinent to viral aerosols. The work so far has provided a sound experimental basis for a broad approach to the problem of the role of viral aerosols in human respiratory disease, and the information already gained has indicated a possible significance for this mode of dissemination of these infections.

RESULTS

Preparation and Properties of a Small-Particle Viral Aerosol

Studies with artificially prepared small-particle aerosols were undertaken to provide better control of the site of inoculation than was possible with liquid suspensions instilled into the noes. Opportunity was also provided to make observations on virological and physical properties of this form of viral suspension. The results to date are limited to findings with coxsackievirus A, type 21, but the methodology is applicable to agents belonging to three other major virus groups: adenoviruses, rhinoviruses, and influenza viruses.

An aerosol apparatus originally designed for use with a bacterial organism (5, 8, 11) and the Collison atomizer (2, 9) were selected for evaluation. The aerosol was generated from a safety-tested, tissue culture suspension of virus (4, 10). The equipment produced a heterogeneously sized, small-particle aerosol under the conditions in which it was used. The sampling instrument used in these studies was the Shipe impinger (16). It contained 5 to 10 ml of a suitable cell culture medium that could be used directly in the selected assay system. The high efficiency of the Shipe impinger for the collection of virus from these aerosols has been established. About 50% of the total virus atomized was recovered.

Preliminary experiments were performed to determine the relationship between the concentration of the viral suspension to be sprayed and the viral concentration of the resulting aerosol. This information was essential to provide a degree of control over doses of virus to be administered. Figure 1 shows data collected with coxsackievirus A-21. It is apparent that a direct relationship exists between the concentration of the virus in the spray suspensions and that of the aerosol. With this information, it was possible to estimate, within an acceptable range, doses of virus to be administered to volunteers by appropriate dilution of the spray suspension. The actual dose administered was determined at the time of each inoculation (4).

Another factor of concern with both the experi-

mental and natural aerosols was the distribution of virus in aerosols of heterogeneous particle size. It was important to know whether virus concentration followed the volume distribution of the aerosol or whether some unknown selective force caused an unexpected concentration of virus in particles of one size or another. To answer this question, the concentration of virus was measured in aerosol particles of various size ranges. The particle-size distribution of the aerosol was determined by direct microscopic measurement, and virus was collected in an Andersen sampler (1). The plates were prepared by pouring a 21-ml base layer of hard agar and, after this solidified, an overlay of 6 ml of 12% gelatin was added (6). The agar served to place the gelatin surface at the proper level below each sieve plate. After sampling, the gelatin in the plates was liquefied at 37 C and was removed for virus assay. Figure 2 shows the results of one of these experiments. As can be seen, the virus concentration appeared to be more closely related to the volume distribution rather than the particle number distribution of the aerosol. Similar findings (1) have been reported for bacterial aerosols.

Particle sizing of virus aerosols, both experimental and natural, presented no unique problems. Standard techniques with use of cascade impactors, membrane filters, and settling slides were used without modification (14).

Viral Aerosols Produced by Infected Persons

For present purposes, natural aerosols are defined as those arising directly or indirectly from infected volunteers. The events that were considered to be possible sources of viral aerosols

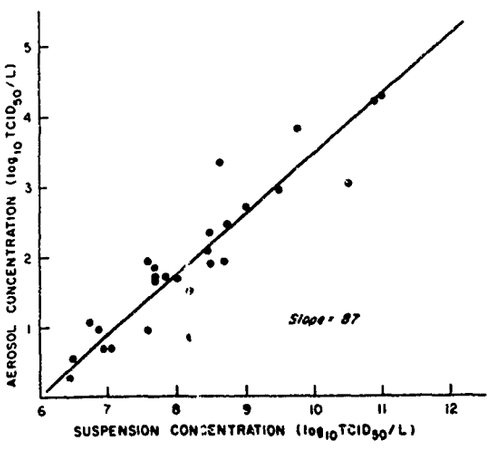

FIG. 1. *Relationship of coxsacki virus A-21 concentrations in spray suspensions and aerosols. Reproduced by permission from reference (4).*

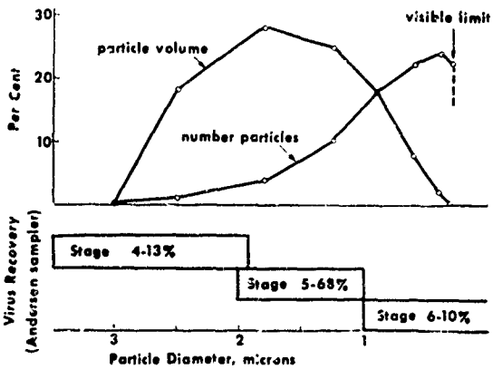

FIG. 2. *Distribution of coxsackievirus A-21 in an aerosol heterogeneous in particle size. Reproduced by permission from reference (4).*

included sneezes, coughs, talking, and breathing. Because talking and breathing produced relatively few particles, our studies were concentrated on the sneeze and cough.

Two procedures were devised to examine the aerosols produced in coughs and sneezes by infected volunteers. One was used to recover virus from coughs and sneezes, whereas the second was principally concerned with sizing and distribution of particles in the aerosol.

Recovery of virus from aerosols and droplets produced by coughs and sneezes was accomplished by having the volunteer sneeze or cough into a deflated weather balloon (Fig. 3). The balloons were washed several times to remove as much talc as possible. They were sterilized while submerged in buffered saline and then stored in a refrigerator. Prior to use, the excess fluid was removed and replaced with 10 ml of cell culture medium. The balloon was attached to a face mask that provided a tight fit around the nose and mouth of the volunteer. After the volunteer sneezed or coughed, the neck of the balloon was clamped off. By use of a Shipe impinger, the air phase of the balloon was immediately sampled. The inlet on the critical orifice was modified from the usual blunt-end capillary to a funnel shape to reduce the loss of larger particles (>5 μ) by impaction (12). The balloon was reinflated with laboratory air, and the wall inside was carefully rinsed with 10 ml of medium. The impinger fluid was assayed for virus directly. The wash medium from the balloon was clarified by centrifugation, and the supernatant fluid was assayed for virus. This procedure gave the approximate amount of total virus in a sneeze or cough, and roughly defined the airborne component as distinct from the portion that either impacted on the inner wall of the balloon or immediately fell out because of large-particle size.

FIG. 3. *Use of a weather balloon for the entrapment of sneezes and coughs.*

Some examples of results obtained by use of this technique on volunteers infected with coxsackievirus A-21 are given in Tables 1, 2, and 3. These results are presented to illustrate that the procedure can be used for detecting virus in these expiratory events. Although the quantities of virus recovered range from a few $TCID_{50}$ to several thousand, the results cannot be considered in absolute quantitative terms. There is little doubt, however, that virus can be aerosolized in the process of sneezing or coughing, and that, in some instances, sufficient quantities are expelled which could account for infection of susceptible individuals in the environment.

Particle-size analyses were made on sneezes and coughs collected in a 127-liter stainless-steel chamber. The chamber was shaped as a truncated cone to minimize impaction of particles on its sides (Fig. 4). It was equipped at the small end with a pneumatic tube that tightly fit the facial contour around the nose and mouth. At the opposite end of the chamber were several sampling ports that would accommodate impingers, impactors, Andersen samplers, and a particle-size analyzer (13). A large weather balloon could be inserted into the chamber with its mouth open to the outside. This balloon would inflate as the aerosol was sampled, avoiding the dilution of the aerosol with outside air. Preliminary particle-size analyses showed that the particle content of room air obscured the particles produced by the sneeze or cough. To circumvent this problem, the volunteer was placed in a plastic tent that was continuously purged with filtered air, as was the chamber. After several minutes of deep breathing in this environment, the particles were almost completely removed and reliable measurements could be made.

An example of the particle-size distribution of aerosols from sneezes and coughs, by use of this equipment, is shown in Table 4. In comparing the sneeze and cough from a single volunteer, it may be noted that the particle-size distributions were similar. The sneeze produced 18 times more particles than did the cough. The volume of the sneeze was about 30 times that of the cough.

Particles above 15 μ in diameter presented a special problem which has not been successfully solved. Because of their high settling rate and low concentration, no attempt was made to enumerate these particles.

Air Sampling in the Environs of Infected Volunteers

After it was established that the infected human volunteer did produce airborne virus, it was of interest to determine whether virus could be recovered from the room air surrounding the subjects. Preliminary calculations were based on the average volume of oral secretions in a sneeze, the expected titer of virus in oral secretions, and the volume of the room. If volunteers harbored 10^4 $TCID_{50}$ of virus per milliliter of oral secretions, sneezed 100 times in a closed room (70,000 titers in volume), and atomized 5.9×10^{-6} ml of secretions with each sneeze, 12,000 liters of air would have to be sampled to recover 1 $TCID_{50}$ of virus. Any biological and physical losses of airborne particles would tend to increase the volume of air that must be sampled. It was apparent, therefore, that devices that sampled 10 to 30 liters of air per minute were impractical for use in these studies. This eliminated from consideration virtually every commonly used sampling device.

The apparatus that was selected for these studies was a newly developed large-volume sampler (LVS; designed by Litton Systems, Inc., Minneapolis, Minn., under contract with Fort Detrick) that functioned by electrostatic precipitation (Fig. 5 and 6). It is capable of drawing air flows up to 10,000 liters per minute. The air passes through a high-voltage corona that charges particulate matter, causing it to precipitate on a grounded disc. The disc rotates at 200 to 300 rev/min and is covered with a thin, flowing film of collecting fluid. The diluent used in

TABLE 1. *Recovery of coxsackievirus A-21 from coughs of volunteers by use of the balloon technique*

Volunteer no.	Source	$TCID_{50}$ of virus							Positive tests[b]
		4 days[a]	5 days	6 days	7 days	11 days	14 days	29 days	
1	Air	30	48	25	0	25	10	0	6/7
	Wall	0	0	260	30	0	0	0	
2	Air	90	0	0	0	0	0	—[c]	1/7
	Wall	0	0	0	0	0	0	—	
3	Air	0	0	0	0	0	0	—	1/7
	Wall	0	0	0	30	0	0	0	
4	Air	90	0	0	10	0	0	0	2/7
	Wall	0	0	0	0	0	0	0	

[a] Days after exposure.
[b] Number of positive coughs/total tested.
[c] Not tested.

TABLE 2. *Shedding of coxsackievirus A-21 by human volunteers*

Volunteer no.[a]	Specimen	$TCID_{50}$					Positive/total[c]
		3 days[b]	4 days	5 days	6 days	7 days	
1	Oral secretion[d]	>32,000[d]	30	100	3,200	100	5/5
	Cough						
	Air[e]	90	0	0	0	0	1/5
	Wall[e]	30	0	0	0	0	
	Sneeze						
	Air	—[f]	0	0	0	—	2/3
	Wall	—	0	30	15	—	
2[g]	Oral secretion			0	100	3,200	2/3
	Cough						
	Air			5	15	0	2/3
	Wall			0	0	0	
	Sneeze						
	Air			0	0	90	1/3
	Wall			0	0	800	

[a] In a third volunteer, all specimens were negative (not infected).
[b] Days after exposure.
[c] Number of positive specimens/total tested.
[d] $TCID_{50}$ per 0.2 ml of secretion.
[e] Balloon technique (see text).
[f] Not tested.
[g] Began shedding virus on day 5.

our experiments was Eagle's basal medium containing 20% calf serum, and antibiotics to reduce bacterial and fungal contamination. About 125 ml of medium was recirculated through the apparatus. Evaporation over a 3.5 min period caused a loss of about 25% of the fluid.

Preliminary tests to determine the efficacy of the sampler were carried out in a room with a volume of 32,800 liters. A suspension of coxsackievirus A-21 was atomized into the room by a University of Chicago Toxicity Laboratories (UCTL) atomizer (15), and the aerosol was circulated by a 15-inch fan directed toward the aerosol stream at a 90° angle (Fig. 7).

Since most determinations were made on aerosol concentrations below the threshold of other sampling devices, there was no base line for comparison. It was necessary, therefore, to calcu-

TABLE 3. *Shedding of coxsackievirus A-21 by human volunteers*

Volunteer no.	Specimen	$TCID_{50}$					Positive total[b]
		3 days[a]	4 days	5 days	6 days	7 days	
1	Oral secretion	10,000[c]	1,000	1	10	100	5/5
	Cough						
	Air[d]	10	150	0	0	0	2/5
	Wall[d]	0	400	0	0	0	
	Sneeze						
	Air	—[e]	0	0	0	0	0/4
	Wall	—	0	0	0	0	
2[f]	Oral secretion		0	100	10	0	2/4
	Cough						
	Air		10	480	0	0	2/4
	Wall		0	80	0	0	
	Sneeze						
	Air		0	4,800[g]	0	9	2/4
	Wall		0	500,000[g]	0	1,600	
3[h]	Oral secretion				1	10	2/2
	Cough						
	Air				15	0	2/2
	Wall				160	30	
	Sneeze						
	Air				—	0	0/1
	Wall					0	

[a] Days after exposure.
[b] Number of positive specimens/total tested.
[c] $TCID_{50}$ per 0.2 ml of secretion.
[d] Balloon technique (see text).
[e] Not tested.
[f] Began shedding virus on day 4.
[g] Gross nasal secretions were expelled by the sneeze.
[h] Began shedding virus on day 6.

late the efficiency of the apparatus from the amount of virus atomized. Figure 8 shows the results of these experiments. Recoveries ranged as low as 0.6% to as high as 71%, with the vast majority falling between 1 and 20%. It is significant that virus was recovered in all experiments in which the predicted aerosol concentration was 0.001 tissue culture infectious unit (TCIU) per liter or greater. [Concentration was estimated by the dilution method of Fisher and Yates (7).]

In trying to establish the best method for handling the fluid from the LVS prior to assay, a number of techniques were employed in an effort to concentrate the virus and reduce the problem of contamination. These included both high- and low-speed centrifugation, sonic disruption, extraction with trichlorotrifluoroethane, and sometimes no treatment at all. Although these procedures were more or less successful in reducing contamination or reducing the volume of fluid to be tested, they did not seem to alter the per cent recovery.

In the interpretation of these recovery values, several factors must be considered:

(i) The sampling period was based on one turnover of room air through the sampler. Since the effluent air was returned to the room, the maximal efficiency would not be expected to exceed 66%.

(ii) No measurement of biological or physical loss of the aerosol was made. Any losses of this nature would reduce the maximal per cent recovery that would be expected.

(iii) When contamination of the cell cultures occurred, the tubes were eliminated from the assay, and it was noted that a low recovery value was obtained in these instances.

A second series of experiments was done in a similar manner, except that a tracer, sodium fluorescein, was incorporated into the virus suspension to be atomized, and large concentrations of virus were used. With these large concentrations of virus, it was possible to make direct comparisons between the LVS and the Porton all-glass impinger (AGI), a common laboratory

FIG. 4. *A stainless-steel, 127-liter chamber for the collection of sneezes and coughs.*

TABLE 4. *Airborne portion of a representative sneeze and a representative cough*

Particle diam	Sneeze		Cough	
	No. of particles	Vol	No. of particles	Vol
μ		μ³		μ³
0.1–1	800,000	167,000	66,000	13,860
1–2	686,000	1,210,000	21,900	37,701
2–4	101,000	1,427,000	2,800	39,564
4–8	16,000	1,800,000	700	79,100
8–16	1,600	1,270,000	38	30,248
Total	1,604,600	5,874,000	90,838	200,473

Ratio of number of particles in a sneeze to number of particles in a cough was 17.6:1; the ratio of volume of a sneeze to volume of a cough was 29.3:1.

aerosol sampler. The LVS was operated for a 3.5-min period, whereas the AGI were operated for 1 min (12.5 liters per minute of flow). Based on the total amount of virus and fluorescein aerosolized into the room and the amounts recovered

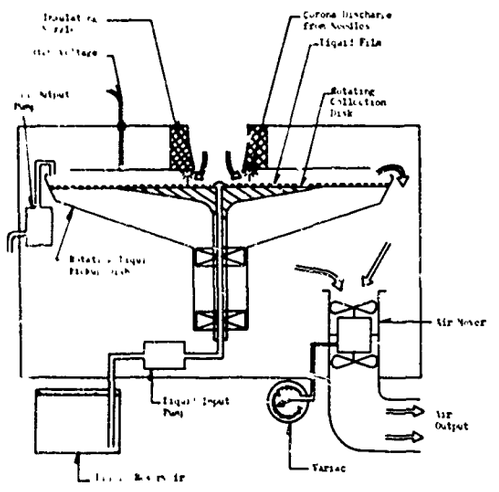

FIG. 5. *Schematic diagram of the air and liquid flow systems of the large-volume air sampler.*

in the samplers, recovery rates were calculated. Table 5 shows that the LVS consistently recovered more fluorescein than the AGI. The virus recovery

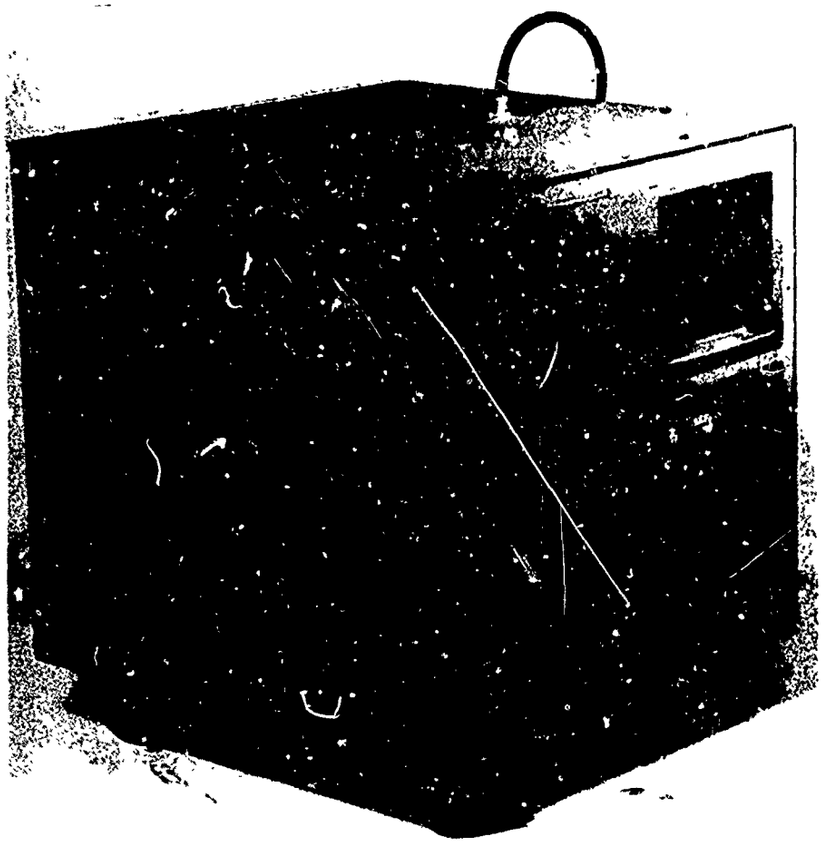

FIG. 6. *Photograph of a large-volume air sampler.*

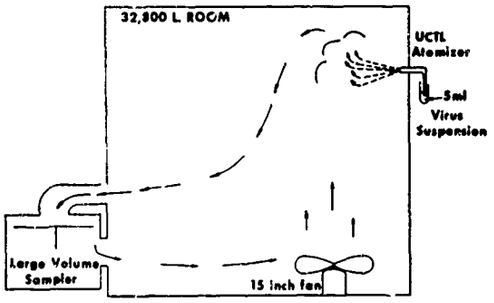

FIG. 7. *Sampling arrangement for testing the efficiency of the large-volume air sampler.*

rates exhibited variability between samples. It was also significant that the recovery rates of the samplers were not changed in situations where sampling was started after the aerosol generator was stopped. These results suggest that the LVS is a highly efficient sampler and that biological inactivation of the virus did contribute to the low recoveries in earlier experiments (Fig. 8).

The large-volume sampler was used for the detection of virus in the air of rooms occupied by volunteers experimentally infected with aerosols of coxsackievirus A-21. Prior to sampling, the

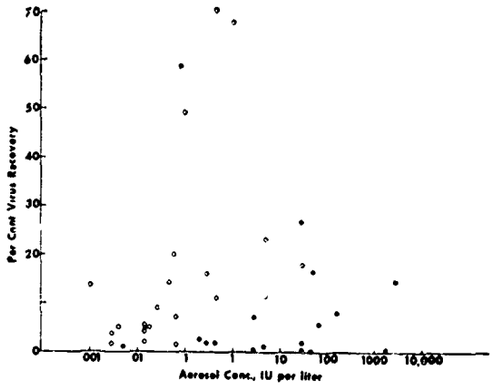

FIG. 8. *Recovery of coxsackievirus A-21 from aerosols of varying concentrations by use of the large-volume air sampler.*

ventilation was turned off for a 2- to 4-hr period. The room was closed except for entry for the sampling. During the 2- to 4-hr period, no restrictions were imposed on the volunteers, and routine activity was normal. The sampler was operated for a 12-min period, which amounted to sampling 120,000 liters of air. The room volume was 70,000 liters. It was estimated that about 82% of the room air was sampled by this procedure. The sampling fluid was immediately frozen and stored for subsequent assay in cell cultures.

The results of one experiment in which two rooms were sampled twice daily for 5 days are shown in Table 6. Virus was recovered from 5 of these 16 samples tested. Overall recovery rates revealed a distinct relationship between the quantity of virus in secretions and recovery of virus in the LVS (3).

DISCUSSION

The purpose of these studies was to describe procedures employed in studies on the role of viral aerosols in human viral respiratory disease. The results showed that viral aerosols prepared with the Collison atomizer can be adjusted to a desired content of virus, and that the size distribution of such aerosols coincides to most particles produced in sneezes and coughs from infected volunteers. Thus, the convenience and precision of the technique and its resemblance, at least in part, to natural viral aerosols indicate its potential utility for studies of this kind.

Virus was recovered from coughs and sneezes by collection in a weather balloon. The disadvantages of this procedure were that only a rough approximation of airborne virus could be obtained and that it was not practical to measure the size of the airborne particles.

The particle-size studies were best performed in a rigid, stainless-steel chamber. These were accomplished by a combined use of a cascade impactor and a particle-size analyzer. The larger particles were not measured by these procedures, because they did not remain airborne long enough and because they were present in relatively low concentrations.

The use of a large-volume sampler to detect virus aerosols in room air proved to be useful, and the presence of virus in the environmental air of infected subjects was demonstrated. When these studies were performed, the apparatus was used essentially as it was originally designed. It is conceivable that, with additional work and modifications, the LVS can be used for quantitative determinations of airborne virus in a natural environment. In this regard, it was of interest to find that the greatest number of positive LVS samples occurred in the room with patients that shed the larger amount of virus (3). With due regard to the inefficiency of present recovery methods, evidence given here and from another study from this laboratory (4) suggests that infected persons may discharge sufficient virus into their environment to account for airborne transmission of this disease.

TABLE 5. *Recovery of coxsackievirus A-21 and fluorescein from room aerosols*

Expt no.	Conditions of sampling	Sampler	Per cent recovery	
			Virus	Fluorescein
7	During spraying	LVS	1.2	64
		AGI_1	2.5	46
		AGI_2	6.0	45
		AGI_3	2.5	41
8	After spraying	LVS	16.0	64
		AGI_1	0	42
		AGI_2	22.0	43
		AGI_3	16.5	39
11	After spraying	LVS	18.8	74
		AGI_1	2.5	42
		AGI_2	3.0	47
		AGI_3	2.5	47
12	During spraying	LVS	7.0	65
		AGI_1	5.4	46
		AGI_2	3.0	52
		AGI_3	2.5	50
Avg		LVS	10.75	66.8
		AGI	7.13	45.0

TABLE 6. *Recovery of coxsackievirus A-21 from room air by use of the large-volume sampler*

Room no.	Sampling time	$TCID_{50}$ of virus by days after exposure				
		3	4	5	6	7
211	7:00 AM	—[a]	0	185	5	0
	10:00 PM	0	0	0	0	—
	No. positive/ no. tested[b]	1/3	1/3	2/3	2/3	2/3
215	7:00 AM	—[a]	0	0	90	90
	10:00 PM	0	5	75	0	—
	No. positive/ no. tested[b]	1/3	2/3	3/3	3/3	3/3

[a] Not done.

[b] Number of volunteers having virus-positive saliva, cough, or sneeze, or all three, over total in the room.

The full significance of these studies will not be realized until investigations of this nature are extended to other respiratory virus diseases. By examining viruses of varying epidemic potential and comparing such factors as infectious dose, clinical illness, virus-shedding patterns, airborne survival, etc. on a quantitative basis, a better knowledge of the underlying mechanisms of airborne transmission of virus will be gained. This information will be helpful in approaches to environmental control of respiratory disease.

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